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# MICRODISSECTION STUDIES, III. SOME PROBLEMS IN THE MATURATION AND FERTILIZATION OF THE ECHINODERM EGG.

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This paper is a record of operative work on the starfish, sea-urchin and sand-dollar eggs to ascertain the morphological nature of changes which take place in the egg during its maturation and fertilization. Results were obtained on the effect of nuclear material on cytoplasm, the nature of cortical changes in the maturing and fertilized egg and the difference between cortex and medulla of the egg with respect to fertilizability and to other life activities. The dissection and injection of the living eggs were carried out at first by means of Barber's ('14) apparatus and later with an improved micromanipulator of my own design ('21<sup>b</sup>). A description of the technique as applied to microdissection has already been published (Chambers, '18<sup>a</sup>). A detailed description of the new micromanipulator will appear both in the *Journal of Bacteriology* and in the *Anatomical Record*.

## I. THE GERMINAL VESICLE IN THE MATURING STARFISH EGG.

Starfish eggs, on being shed naturally, have already begun maturing. In order, however, to secure large quantities of eggs, it has been the general custom to remove the ovaries bodily from a ripe female and to cut them up in a bowl of sea water. This procedure brings the eggs into the sea water in the immature condition with germinal vesicles intact. The germinal vesicle begins to disappear anywhere from thirty to fifty minutes after the eggs come into contact with the sea water and maturation usually proceeds in a normal manner (Wilson and Mathews, '95).

The undisturbed germinal vesicle or nucleus of a fully grown

immature egg is a hyaline sphere containing a sharply differentiated nucleolus and occupying about one fifth the volume of the egg. With the microdissection needle the vesicle may be moved about in the fluid cytoplasm without injury to the egg. With the needle one may considerably indent the surface of the vesicle. On removal of the needle the vesicle reverts again to the spherical shape (Fig. 1). The vesicle possesses a morphologically definite surface membrane inclosing an optically homogeneous liquid (cf. Chambers, '18<sup>b</sup>). Within this liquid lies a visible body, the nucleolus. By agitating the vesicle the nucleolus may be made to occupy any position within the nuclear fluid. The nuclear membrane is very easily injured. If, however, a microneedle be carefully inserted into the nucleus, the membrane about the puncture adheres to the body of the needle and the tip of the needle may push the nucleolus about with no apparent injury. The existence of considerable tension in the nuclear membrane is shown in the following experiment. An egg was cut into three fragments in such a way that the surface film forming over the cut surfaces of the middle fragment pressed upon the nucleus, deforming it considerably (Fig. 2). The attempt of the nucleus to return to a spherical shape bulged out one end of the egg fragment until it was constricted off from the remainder of the fragment (Fig. 2*b-f*).

Tearing the nuclear membrane in most cases results in a destruction of the nucleus. In a few cases it was possible to produce a slight rupture with no noticeable injurious effects. Such a case is recorded in Fig. 3. At 10:44 A.M. undue pressure on the germinal vesicle when cutting an immature egg in two resulted in its rupture followed by a lobular extrusion bounded by a very delicate film. During the following ten minutes the vesicle began slowly to revert to its original shape (Fig. 3*b* and *c*). Before that was attained the maturation process began and, at 10:55, the outline of the vesicle had disappeared. The nucleated egg fragment matured normally and five hours and a half after insemination it had segmented in two. At 8:40 P.M. it had developed into a swimming blastula.

The cytoplasm of the egg allows of considerable tearing without

apparent injury (Chambers, '17-a). If, however, the nuclear membrane be torn, a very striking phenomenon occurs. The cytoplasm immediately surrounding the nucleus disintegrates and

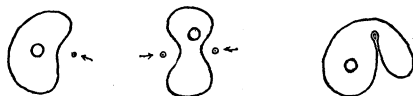


Fig. 1

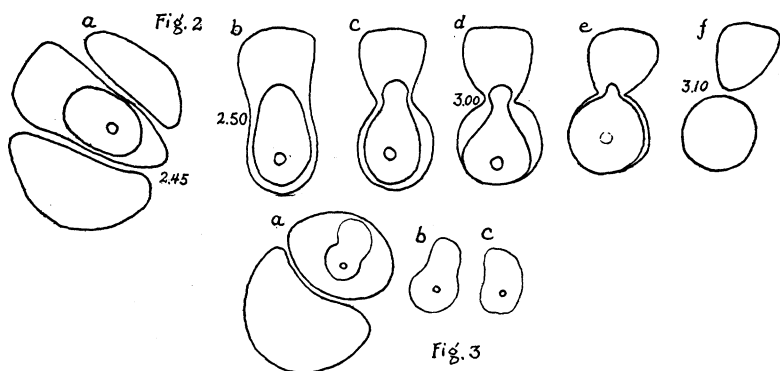


Fig. 3

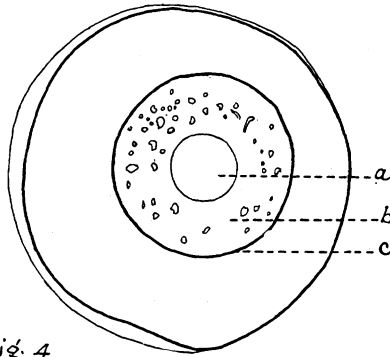
FIG. 1. Figures showing the extent to which the nucleus (germinal vesicle) of an immature starfish egg may be indented on one or both sides without rupture. On removing the needle the nucleus reverts to its original spherical shape.

FIG. 2. *a*, immature starfish egg cut at 2:45 P.M. into three parts; the nucleus has remained intact but is laterally compressed in the middle fragment. *b*, *c*, *d*, *e* and *f*, successive steps in attempt of nucleus to round up; *b*, 2:50 P.M.; *d*, 3:00 P.M.; *f*, 3:10 P.M.

FIG. 3. *a*, partial rupture of nucleus followed by a repair of its membrane. *b* and *c*, successive changes in the shape of the nucleus within the following ten minutes after which time it disappeared.

liquefies. If the rupture of the nucleus be violent, the disintegration of the cytoplasm spreads rapidly until the entire egg is involved. If the rupture be slight, the disintegrative process is quickly limited by a surface film which forms on the boundary between the disintegrating and the surrounding healthy cytoplasm (Fig. 4). This film tends to prevent any further spread of the destructive process. The destruction of the cytoplasm is evidently due to something which emanates from the injured nucleus. The injury to the cytoplasm does not start where the nuclear membrane is first torn, but from the entire surface of the injured nucleus.

This is analogous to results obtained by injuring red blood corpuscles with a needle upon which hemoglobin escapes immediately from the entire surface (Chambers, '15).



*Fig. 4*

FIG. 4. Disintegration of cytoplasm surrounding the nucleus on tearing the nucleus with a needle. (a) Faint hyaline sphere, a remnant of the destroyed nucleus. (b) Disintegrated cytoplasm. (c) Cytoplasmic surface film separating disintegrated from healthy cytoplasm.

Within the nucleus itself the immediate effect of the injury is a dissolution of the nucleolus. A nuclear remnant tends to persist after the injury as a hyaline sphere lying within the disintegration products of the cytoplasm. On being touched with the needle it fades from view.

In permanently immature eggs, such as eggs which have been standing in sea water for an hour or more without maturing, the disintegrative effect on the cytoplasm by injuring the nucleus tends to be much more restricted, and the nuclear sphere which persists after the injury can be shown to possess a morphologically definite membrane. Such a sphere is easily dissected out of the egg. Frequently, when the germinal vesicle lies close to the periphery of the egg, the disintegration of the cytoplasm quickly reaches the surface. With the formation of a surface film over the healthy cytoplasm the disintegrative area lies in a deep bay on one side of the egg. This hollow is slowly obliterated as the semi-fluid substance of the egg strives to assume a spherical shape. In this way the disintegrated material is forced out of the egg together with the persisting nuclear sphere. This nuclear sphere persists for some time in the sea water. It can be deformed by means of the needle and, on

tearing its surface, the fluid contents escape, leaving behind a collapsed membrane which disappears within 10 to 15 seconds.

Fig. 5 shows the effect of cutting the mature egg nucleus of the starfish egg. By pushing the nucleus against the inner surface of

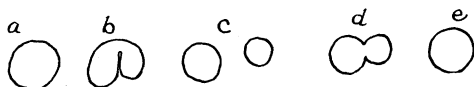


Fig. 5

FIG. 5. Effect of cutting mature nucleus of a starfish (*Asterias*) or sea-urchin (*Arbacia*) egg. *a*, intact egg nucleus; *b*, nucleus in process of being cut in two. The nucleus was pushed against the periphery of the egg as it was being cut by a vertical needle; *c*, the separated fragments of the nucleus; *d*, reunion of the fragments; *e*, reconstituted nucleus.

the egg it is possible to pinch it into two pieces. Each piece rounds up but, if the two are allowed to come into contact, they will fuse into a single nucleus again. The same result obtains in the sand-dollar and sea-urchin eggs. If, however, the nuclear membrane be torn, a disintegration of the cytoplasm results analogous to that produced on rupturing the germinal vesicle. The extent of disintegration is much more limited, owing doubtless to the much smaller amount of nuclear material present. Similar results were obtained on tearing the nucleus of the *Arbacia* egg.

It was found possible to destroy the cytoplasm of one egg by injecting into it nuclear material obtained from another egg. This experiment has to be performed very rapidly, for if the nuclear material be allowed to remain longer than ten seconds within the pipette it has no effect whatever when injected into the cytoplasm of an egg. If it be injected within that time the destructive effect is very pronounced.

If an egg be allowed to undergo normal maturation, the germinal vesicle disappears except for a small remnant which becomes the definite egg nucleus. This egg nucleus moves to the surface of the egg, where it gives off the two polar bodies. It then constitutes the female pronucleus, which remains quiescent until fertilization occurs. The disappearance of the germinal vesicle is a well-known phenomenon. In order, however, to locate definite stages selected for my operations I introduce the following sum-

mary. The germinal vesicle with an intact membrane is shown in Fig. 6. Within thirty to forty-five minutes after standing in sea water the nuclear membrane exhibits wrinkles and its outline begins to fade from view. Within a few minutes no membrane is visible and cytoplasmic granules can be seen moving into the region hitherto occupied by the nucleus, while the nuclear sap appears to be diffusing out (Fig. 6-c). As the nuclear membrane disappears the nucleolus fades from view. The invasion of the nuclear area by cytoplasmic granules continues until all of the area except a small portion is rendered indistinguishable from the general cytoplasm of the egg. This small portion persists as the egg nucleus (Fig. 6e and f). In Fig. 6-g two consecutive positions of the nucleus are shown. At 1:13 P.M. it lay deep in the substance of the egg. In twenty minutes it had moved to the periphery of the egg preparatory to the formation of the polar bodies.

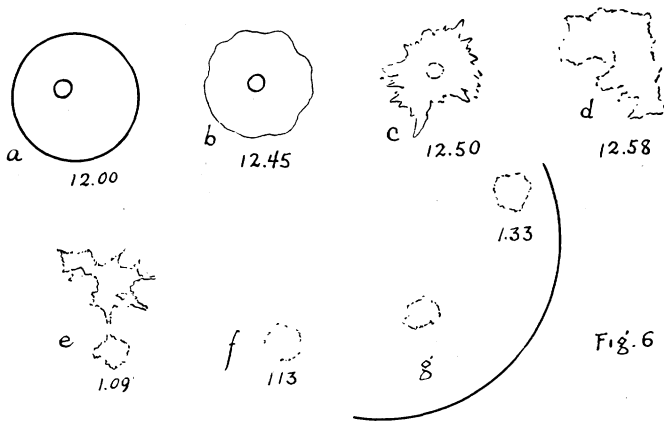


Fig. 6

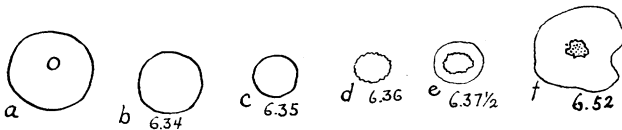


Fig. 7

FIG. 6. Camera lucida drawings of the successive steps in the normal dissolution of the germinal vesicle in the maturing starfish egg. The process was somewhat slowed down owing possibly to the compressed condition of the egg necessary for detailed observation.

FIG. 7. a, intact germinal vesicle within the egg. b, nucleus after having been torn out of the egg and brought into sea water. c, d, e and f, successive changes undergone by the nucleus lying in sea water.

By means of the microdissection needle it is possible to show, at the stage shown in Fig. 6-*d*, that the membrane of the germinal vesicle no longer exists. By careful manipulation it was possible to push the cytoplasmic granules into the nuclear area. A slight rapid movement of the needle, however, was sufficient to give rise to disintegrative processes similar to those on tearing an intact germinal vesicle. In the normal maturation process the mingling of the nuclear sap with the cytoplasm is very gradual, being completed in the case recorded not under ten minutes. It is this gradual mixing which apparently prevents disintegration.

Morgan ('93) and Mathews (Wilson and Mathews, '95) found that maturation was accelerated by shaking starfish eggs shortly after they were placed in sea water. They concluded that the shaking ruptured the membrane of the germinal vesicle and so allowed the nuclear material to mix more quickly with the cytoplasm. I have repeatedly tried to intermix cytoplasm and nuclear material by rupturing the nuclear membrane of the starfish egg with the needle, but in every case I get an explosive disintegration of the cytoplasm. The ruptured nuclear membrane which Mathews (W. and M., '95) and Marcus ('07) describe in fixed and stained immature eggs which had been violently shaken is possibly the membrane of the sphere which I found to persist after injury to the germinal vesicle (see page 321). It is more likely that the shaking which accelerates processes within the egg leads to the normal gradual dissolution of the nuclear membrane and the subsequent diffusion of the nuclear material throughout the egg. I have been able to do this occasionally with the needle. An intact germinal vesicle which to all appearances should take fifteen to twenty minutes to go into dissolution will often immediately exhibit a wrinkled outline on being gently agitated with the needle. Then follows the gradual fading from view of its outline with the subsequent changes as shown in Fig. 6.

The intact germinal vesicle may be brought into the sea water by tearing away the surrounding cytoplasm. During the process the nucleolus fades from view. The slightest tearing of the nuclear surface then causes the entire liquid vesicle to disappear in the water. If, however, the nucleus be left alone, it shrinks for a



time and then swells. The changes appreciable to the eye are shown in Fig. 7. During the swelling of the nucleus a substance apparently separates out which collects into a small mass and persists as a gelatinous body. It is possible that this abnormal separating out is analogous to the formation of the definitive egg nucleus in the normal process of maturation. This separating out of a gelatinous material from a liquid nucleus upon injury may be similar to the method of precociously inducing chromosomes in spermatocytes of the grasshopper (Chambers, '14).

## 2. THE EXISTENCE OF AN EXTRANEOUS MEMBRANE ABOUT THE UNFERTILIZED EGG.

The existence of a membrane about the unfertilized egg rising off as the fertilization membrane upon insemination was first suggested by the earlier investigators (*e.g.*, Hertwig, '76; Herbst, '93). Kite ('12) and Glaser ('13) agreed with them whereas McClendon ('14), Harvey ('14) and Elder ('13) claimed that the fertilization membrane is a new formation consequent to fertilization. Heilbrunn ('13) also identifies it with the actual protoplasmic surface of the egg, which he considers to be in a state of a gel and which lifts off as the fertilization membrane, a new surface film forming over the egg underneath it.

My experiments indicate that the unfertilized eggs of the starfish, sea-urchin and sand-dollar all possess a membrane extraneous to their true protoplasmic surface, and that it is this membrane which, upon insemination, is lifted off as the well-known fertilization membrane.

In the unfertilized egg the membrane is more or less tightly glued to the surface of the egg just as Kite ('12) described it. In the sea-urchin egg it is extremely delicate and can be demonstrated only as follows (Fig. 8): The needle is inserted as nearly as possible through the periphery of the egg and left there. Within a few seconds the protoplasm, lying immediately under the egg membrane and distal to the needle, flow away from the needle until the needle lies in a small protuberance which is formed by a very slightly lifted portion of the egg membrane.

The existence of the egg membrane is easily demonstrated in the

starfish egg. In Fig. 9 the disintegration of the cytoplasm following injury to the germinal vesicle has reached the surface of the egg. The disintegrated area is quickly localized by a surface film bounding a cup-shaped depression on the surface of the egg. Roofing over the depression is the egg membrane. The egg membrane can also be shown by cutting an egg in two by pressing the egg against the coverslip with the side of a needle. The pressure of the needle cuts the egg in two without rupturing the membrane, which, on releasing the egg, bridges the gap between the pieces and holds them together (cf. Figs. 11 and 12, page 329).

The difference between the consistency of the egg membrane in the starfish and the sea-urchin egg is strikingly shown in the fol-

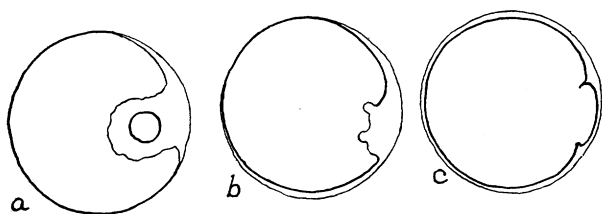
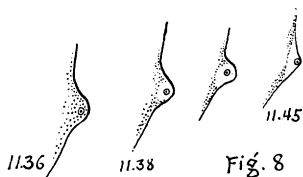


Fig. 9

FIG. 8. Needle inserted at 11:36 A.M. through periphery of a sea-urchin egg and left there. At 11:38 the cytoplasmic granules have been flowing away from the needle. A new surface film begins to appear with the needle left outside. At 11:45 the original egg membrane appears as a delicate membrane partially lifted off the surface of the egg by the needle.

FIG. 9. Lifting of a membrane from the surface of an immature starfish egg following injury to the egg. *a*, local disintegration of cytoplasm following destruction of the germinal vesicle (cf. Fig. 4). An egg membrane becomes apparent as the cytoplasm retreats from it. *b* and *c*, gradual separation of the membrane all over the surface of the egg.

lowing experiments. With the eggs in a hanging drop the egg is pressed against the coverslip with the side of a glass needle until

the pressure divides the egg into two pieces. In the sea-urchin egg the two pieces immediately round up and roll away from one another. In the starfish egg the tougher membrane is not ruptured, but holds the two pieces together.

The membrane of the sea-urchin egg is so delicate that it is also possible to cut the egg in two in the following manner: In a hanging drop the horizontal end of the needle is brought *over* the egg (Fig. 10). The needle is now lowered. This brings the needle

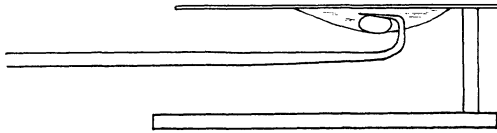


Fig. 10

FIG. 10. Side view of moist chamber to show one method of cutting an egg in two with the microdissection needle.

against the upper surface of the egg and presses the egg down against the surface film of the hanging drop. On lowering the needle still further it passes through the egg and out of the drop, cutting the egg cleanly in two. In the case of the starfish egg this procedure would drag the egg out of the drop along with the needle. The membrane of the sand-dollar egg is weaker than that of the starfish and stronger than that of the sea-urchin egg.

The consistency of the membrane varies with the age of the egg. The full-grown immature egg of the starfish has a relatively tough membrane. On the other hand, young ovarian eggs possess very delicate membranes and they can be cut in two with the same ease as mature sea-urchin eggs.

The strongest argument regarding the existence of a membrane about the unfertilized egg is that a membrane may be stripped off the egg whereupon the egg, which was previously non-adherent, now sticks to everything it touches. The fertilizability of such naked eggs is discussed under the next heading.

The existence of egg membranes is a fairly universal feature and it is, therefore, not surprising that we should find them in the

echinoderm eggs which have generally been considered as naked. The unfertilized *Cumingia* egg has an extremely tough membrane, so tough that it is difficult to rupture it without completely destroying the egg contents. The vitelline membranes in the frog and in the chick are undoubtedly analogous structures.

### 3. THE EGG MEMBRANE AND THE FERTILIZATION MEMBRANE ARE IDENTICAL.

Prior to fertilization no membrane enveloping the egg is visible. Upon fertilization a membrane lifts off which can easily be cut away from the egg. Figs. 11 and 12 indicate the identity of a preexisting membrane with the fertilization membrane. Fig. 11-*a* shows an egg cut in two with an investing membrane holding the pieces together. Upon fertilization the membrane lifts off, enclosing the two pieces in a single cavity (Fig. 11-*b*). One only of the pieces happened to segment, and the fact that the two pieces lie in one cavity is shown in Fig. 11-*c*, where the blastomeres of the segmented portion have encroached on the area around the nonsegmented piece. In Fig. 12 an egg was cut into three pieces, the egg nucleus lying in one of the pieces. Upon fertilization the membrane lifted off the pieces, each of which received sperm and developed into swimming larvæ. Fig. 12-*c* shows the empty fertilization membrane after the three larvæ had escaped. In Fig. 13 is shown an egg which, on being cut in two, was rolled about in an attempt to separate the pieces. The egg membrane between the two pieces was twisted into a thread joining the two. Upon fertilization each piece exhibited a complete fertilization membrane, but the fact that the two investing membranes are portions of one common membrane is shown by the connecting thread.

A conclusive test for the starfish and sand-dollar egg is the removal of the egg membrane prior to insemination. Occasionally, pricking the egg is sufficient to elevate the membrane. No subsequent development takes place. It is possible, however, to remove this membrane by tearing it and the egg then be made to slip out. This is more easily done on eggs which have been standing for some time in seawater. On catching at the sur-

face of such eggs with the needle, the membrane is often torn in such way that the egg slips out leaving the membrane stuck to the needle. Such an egg, when inseminated, is fertilized and subsequently segments with no investing membrane whatever.

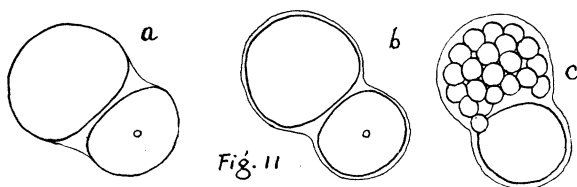


Fig. 11

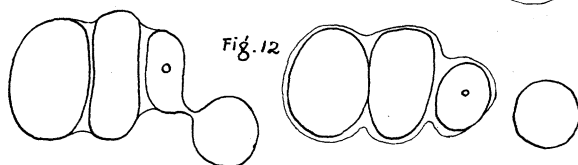


Fig. 12

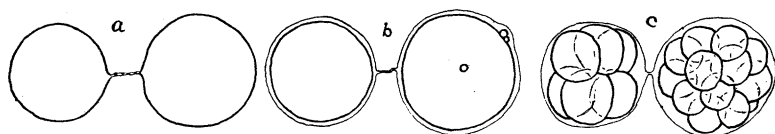


Fig. 13

FIG. 11. *a*, starfish egg cut in two without destroying the investing membrane. *b*, after insemination the investing membrane lifts off both fragments as the fertilization membrane. *c*, one of the fragments segmented, the other did not. That both fragments lie in a common cavity is shown by the encroaching of blastomeres of one fragment into the region of the unsegmented fragment.

FIG. 12. *a*, starfish egg cut into three pieces. One piece was squashed and produced an exovate. *b*, on being fertilized the exovate was pinched off as an endoplasmic sphere (cf. Fig. 25). The rest of the fragments produced a common fertilization membrane. Each of the three enclosed fragments developed into a swimming larva.

FIG. 13. *a*, sand-dollar egg rolled as it was cut in two. The egg membrane between the two pieces was twisted into a thread joining the two. *b*, egg shortly after fertilization showing fertilization membrane about each connected by a filament. *c*, the two pieces in an early segmentation stage.

The difference in reaction of sperm to an egg which has been denuded of its membrane as well as of its jelly, and to one which has not is very striking. An egg within its membrane is quickly surrounded by spermatozoa as they are trapped in the jelly surrounding the membrane. In a membraneless egg no crowding of spermatozoa is noticeable and heavy insemination is necessary

to bring about fertilization. When a cloud of sperm has been blown upon a naked egg, one may frequently observe a spermatozoön swim toward it, wander over its surface, and then swim away. On the other hand, the empty membrane with its investing jelly immediately becomes covered with a halo of spermatozoa. This observation accords with the interpretation of Buller ('02), that the investing jelly determines the direction of the sperm which are captured by it, and that there is no apparent chemotactic substance excreted by the egg to attract the sperm.

The difference in position of the polar bodies in the starfish egg with respect to the fertilization membrane as shown by Gemmill ('12) (see also Chambers and Mossop, '18, and Garrey, '19) may be explained as follows: When the polar bodies form prior to fertilization they rise off the surface of the egg, carrying with them the closely adherent membrane. When they are pinched off the egg membrane remains continuous about the egg and subsequent insemination results in the formation of a fertilization membrane with the polar bodies lying outside. If, however, the eggs are inseminated before extrusion of the polar bodies, the egg membrane lifts off as the fertilization membrane and, when the polar bodies are formed, they lie within the membrane.

In the sea-urchin egg the identity of the egg membrane with the fertilization membrane is more difficult to demonstrate. In Fig. 14 is shown the effect of locally injuring the surface of the sea-urchin egg. In *a* is a disintegrated mass produced by tearing a spot on the surface with a needle. In *b* this area is shown as a bulge which may be explained as being produced by the interior pressure of the egg on a surface weakened by the loss of an investing membrane. In *c* the egg has been fertilized. The fertilization membrane is formed over all the surface except at the injured place. In *d* segmentation has occurred and a blastomere protrudes through the gap in the fertilization membrane.

A better demonstration is the case shown in Fig. 15. At 4:26 the tip of a needle was punched through the cortex. Within a few seconds the cytoplasm distal to the needle flowed away, leaving the needle lying under a delicate membrane (Fig. 15-*a*). At 4:27 the egg was inseminated with the needle still in place. At 4:29

the fertilization membrane was formed, showing its continuity with the delicate membrane previously noticeable (Fig. 15-b).

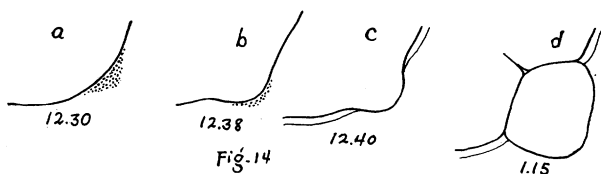


Fig. 14

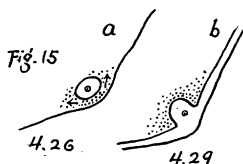


Fig. 15

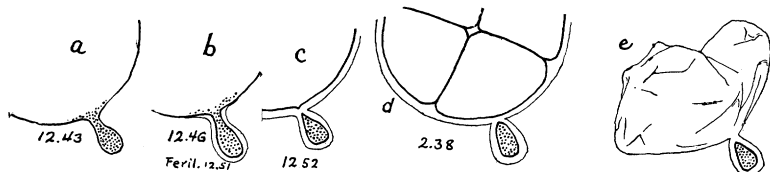


Fig. 16

FIG. 14. Sea-urchin egg with surface torn producing local cytolysis. *a*, a new surface film has formed under the cytolized area which is being extruded. *b*, a bulge appears in the region of the new surface showing this region to be weaker than elsewhere on the egg surface. *c*, egg after fertilization exhibiting a fertilization membrane over the egg except at the place previously torn. *d*, the same egg 35 minutes later with a blastomere protruding through the tear.

FIG. 15. *a*, needle piercing sea-urchin egg near its periphery. The cytoplasmic granules are flowing in the direction of the arrows. One minute later the egg was inseminated. *c*, an intact fertilization membrane forms, inclosing both egg and needle tip.

FIG. 16. *a*, protrusion on surface of egg produced by pulling at cortex with needle. *b*, three minutes later the investing membrane lifted off surface of protrusion. *c*, one minute after fertilization. The protrusion has been pinched off from the egg and its investing membrane can be seen to be continuous with the fertilization membrane. *d*, empty and collapsed fertilization membrane.

In the sea-urchin egg the membrane often rises off a protrusion caused by pulling at the cortex with the needle. Such a case is shown in Fig. 16. The protrusion was formed at 12:43. At 12:46 a membrane had lifted off the protrusion. At 12:51 the egg was inseminated, and one minute later the membrane was

found continuous with the fertilization membrane. The protrusion subsequently pinched itself off and persisted in a sac-like protuberance of the fertilization membrane (Fig. 16-*d-e*).

In all of the various eggs studied a change in the consistency of the membrane takes place very soon after it has been elevated. The membrane, at first very soft and delicate, progressively toughens until it becomes almost parchment-like during the later segmentation stages. It is of interest to note that Harvey ('10) found a difference between the unfertilized and the fertilized sea-urchin egg when subjected to sulfuric acid. The acid dissolves the unfertilized egg completely, whereas it dissolves all of the fertilized egg except the fertilization membrane. Some chemical change apparently takes place as the membrane lifts off the egg.

Outside the membrane is a considerable zone of a structureless jelly. In the sand-dollar egg the jelly very loosely adheres to the membrane. On cutting into the jelly the egg with its membrane easily slips out. This is to a somewhat lesser degree true for the starfish egg. In the starfish egg one often sees the under surface of the jelly pushed away from the surface of the unfertilized egg by the protruding polar body.

The question as to whether the membrane lifts off the surface of the egg or whether the egg shrinks leaving the membrane behind has been raised by Glaser ('14) in spite of McClendon's ('10) statement to the contrary. Glaser, by making a large series of measurements, claims that the egg shrinks upon fertilization, and that the initial diameter of the completed fertilization membrane is equal to that of the unfertilized egg. Glaser's measurements were made on the assumption that the eggs always maintain a spherical shape. This is not true. The mature unfertilized egg is very soft and if allowed to lie on the bottom of a glass dish tends to flatten into the shape of a disc. Upon fertilization the egg rounds up as the fertilization membrane leaves its surface. One can readily see if the observations are taken of eggs in one plane only that erroneous conclusions may be arrived at.

I used two methods to ascertain the diameter of starfish eggs before and after fertilization. One method was to place a drop



containing a few eggs on a gelatin-coated slide. The eggs were rolled over by means of a micro-needle and only those which maintained their spherical shape were measured. With a micro-pipette sperm were introduced into the drop without disturbing the relative positions of the eggs. A second method was to place several eggs in a hanging drop in a Barber moist chamber. By piercing the surrounding jelly with a needle the egg to be measured could be held suspended in the middle of the drop. Numerous measurements of the starfish egg were made at different times through several summers and in every case the egg maintained its original size as the fertilization membrane rose off its surface. Not only does the egg not decrease in volume, but it slightly *increases* in size until segmentation occurs. The accompanying table is one sample of the measurements made:

	Un-fertil.	Minutes after Fertilization.					
		1"	2"	7"	10"	20"	70"
Egg diameter.....	3.4	3.4	3.4	3.4	3.5 x 3.55	3.5 x 3.6	3.5 x 3.6
Fertilization membrane diameter.....		3.5	3.6	3.65 x 3.7	3.65 x 3.7	3.75 x 3.75	3.9 x 3.9

The conclusions from this table apply both to starfish and sea-urchin eggs. They may not necessarily be true for other species.

Fig. 17 shows successive steps in pulling a starfish egg out of its fertilization membrane. No second membrane is ever formed even with superimposed insemination. Occasionally the hyaline plasma layer in such an extruded egg swells up and simulates a second membrane, and it is probably this that has been described by certain investigators as a second fertilization membrane. The hyaline plasma layer will be discussed under heading 5.

An unfertilized mature sea-urchin egg may be rolled about and its contents churned to the extent of producing "fountain currents" within the egg (Chambers, '17-*b*). This is done by pushing an egg in a drop shallow enough to compress the egg. Currents are produced which flow backward immediately under the surface of the egg and forward along its central axis (Fig. 18). By careful manipulation it is possible to do this without rupturing

the investing membrane. Such an egg is capable of forming a normal fertilization membrane when inseminated. If the pushing process be carried too far, a distinctive quiver can be recognized, as of something giving way. On subsequent insemination such

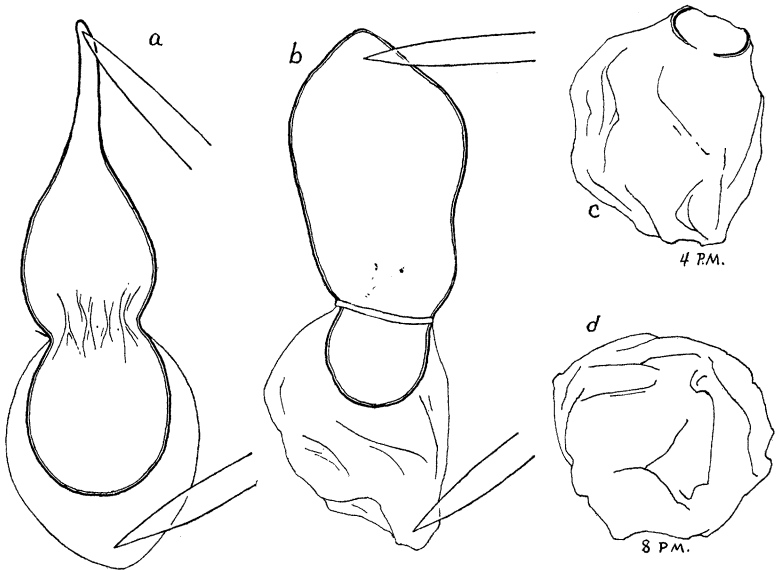


Fig. 17

FIG. 17. *a* and *b*, successive steps in pulling a starfish egg out of its fertilization membrane. *c*, empty membrane at 4:00 P.M. *d*, ditto four hours later at 8:00 P.M. The membrane persists as a collapsed remnant for a long time.

eggs produce a collapsed fertilization membrane. The quiver undoubtedly was due to a rupture of the egg membrane. On account of this rupture the fluid, which presumably collects under the membrane, leaks out and the membrane is not lifted uniformly.

#### 4. THE CORTEX AND INTERIOR OF THE UNFERTILIZED EGG.

The cytoplasm of the immature starfish egg is uniformly semi-solid. A gash made in it with a needle is maintained for some minutes before closing up. When the germinal vesicle breaks down naturally, the egg protoplasm becomes more fluid so that a gash

through such an egg quickly closes up. The cortex—*i.e.*, the surface of the egg immediately beneath the egg membrane—tends always to remain more solid (Chambers, '17-*a*). Because of this difference in consistency the cortex and medulla of the egg can be separated from one another as follows ('21<sup>a</sup>): If the surface of the mature starfish egg be torn with a needle and the egg then be caught at the opposite side and pulled to the edge of the

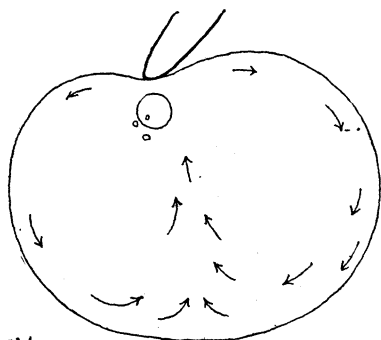


Fig. 18

FIG. 18. Currents produced within a sea-urchin egg by pushing a sea-urchin egg held against a coverslip by a shallow film of water. The direction of the currents is shown by the arrows. The nucleus, after being carried about with the current, tends to come to rest in the location shown in the figure.



Fig. 19

FIG. 19. Part of the cortex of a fertilized egg after the appearance of the hyaline plasma layer. The cortex was ruptured in one place and cytoplasmic granules can be seen issuing through the rupture in the hyaline plasma layer and the investing fertilization membrane.

hanging drop, the compression on the egg produced by the shallow water at the edge of the drop will cause the fluid interior to ooze out through the tear to form a spherical exovate (see Fig. 25, page 344). One may so manipulate the process as to cause the egg nucleus either to remain behind in the cortex (the cortical remnant) or to pass into the extruded sphere of endoplasmic material.

The cortical remnant is relatively solid and remains more or less inclosed within the egg membrane and its jelly. If left long enough it will eventually round up so as to present the appearance of a diminutive egg surrounded by a collapsed and wrinkled egg membrane.

The endoplasmic material which has escaped from the egg into the sea water is fluid and tends immediately to round up. On tearing with a needle its surface behaves like that of a highly viscous oil drop, adheres tenaciously to glass. As long as it possesses an intact surface it looks exactly like an egg fragment and will undergo disintegrative changes similar to those of entire eggs on being torn with the needle (cf. Chambers, '17-a).

The ability to produce endoplasmic spheres is possibly due to the relatively tough egg membrane in the starfish egg which helps to keep back the adherent cortex. In the sea-urchin egg, with an extremely delicate egg membrane, it has been impossible to cause the interior to flow out, as the cortex tends to flow with it.

The sand-dollar egg behaves very much like the starfish egg. The egg membrane is appreciable in the unfertilized egg and endoplasmic spheres are readily produced.

A difference in the functional activities of the cortex and interior of the starfish egg is discussed under the headings 6 and 7.

##### 5. THE HYALINE PLASMA LAYER.

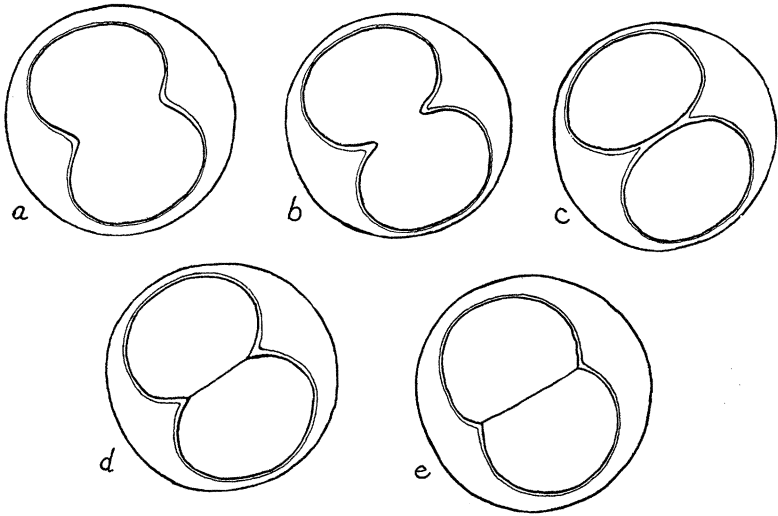
Prior to fertilization the cytoplasmic granules in the sea-urchin and sand-dollar egg lie close to the surface. Within ten minutes after fertilization the granules have undergone a centripetal migration, leaving an appreciable peripheral zone of a hyaline appearance which has been called the hyaline plasma layer (Loeb's gelatinous film, '13, p. 19).

The microdissection needle indicates that this layer is relatively firm and gelatinous. The very fluid internal cytoplasm may be made to flow out through a rupture in this layer if the egg be torn. This is shown in Fig. 19. The cytoplasmic granules lie against the inner boundary of this layer and may be seen oozing out through the small tear in this layer and through a tear in the fertilization membrane to the exterior.

The hyaline plasma layer adheres very tenaciously to the needle and when an egg has been deprived of its fertilization membrane the egg sticks to everything it touches.

Loeb has called attention to the fact that the hyaline plasma

layer in a segmented egg bridges the segmentation furrow. When the furrow is first formed, however, the hyaline plasma layer does not bridge the furrow, but is carried in on the walls of the cleavage furrow (Fig. 20-*a, b, c*). The layer is thicker in the floor of the



*Fig. 20*

FIG. 20. Contour of a sand-dollar egg at various stages of its cleavage into two blastomeres. In *a* and *b* the hyaline plasma layer is seen carried in on the walls of the deepening furrow. In *c* the egg has segmented in two with the hyaline plasma layer on opposite sides of the furrow tending to merge into each other. In *d* this process is carried further. In *e* the two blastomeres are tending to assume the shape of hemispheres with the hyaline plasma layer bridging the furrow.

furrow, but it is only later when the furrow has cut through the egg that the hyaline plasma layers on the opposite surfaces of the furrow run together. Each half of the segmenting egg tends to assume the shape of a sphere owing to the separation of the two asters of the amphiaser (Chambers, '17-*b*, '19). If there were no other forces at play, the two blastomeres, when formed, should be spheres. In the sea-urchin egg the adhesiveness of the hyaline plasma layer tends to draw the two blastomeres together; also the fertilization membrane, not rising to any great extent off the surface of the egg, must exert some pressure on the two blastomeres. In the sand-dollar the fertilization membrane is well

lifted, so that there is plenty of room within the membrane, permitting the two blastomeres to assume almost spherical shapes (Fig. 20-*c*). When the cleavage furrow is completed the two blastomeres are contiguous only where the two spheres touch. At this place the hyaline plasma layers of the two blastomeres merge. We have here, apparently, two opposing forces; first, the jellied aster holding each blastomere to a spherical shape, and, second, the affinity of the plasma layer substance surrounding the two blastomeres. As soon as the asters disappear and the cytoplasm of the blastomeres reverts to a more fluid state the plasma layers of the two blastomeres merge more and more and the blastomeres are pulled together till they assume shapes approaching those of hemispheres (Fig. 20-*e*). The outlines in Fig. 20 are camera lucida drawings taken during the successive stages of one sand-dollar egg.<sup>1</sup>

In the starfish, where there is no appreciable hyaline layer, and where the fertilization membrane is lifted far beyond the surface

<sup>1</sup> It has recently been intimated that the microdissection method is unreliable as a means of ascertaining changes in viscosity in the dividing egg because of supposed discrepancies in the results obtained by Seifríz ('20) and myself ('17" and '19). As a matter of fact the results of Seifríz harmonize perfectly with mine. Seifríz states "there is a pronounced decrease in viscosity of the central region of the cell with the first appearance of the amphiasters." This statement has been interpreted as running counter to mine. This is not true for although my results indicate that the astral portion of the amphiaster is jellied, I definitely state (p. 494, '17) that the central region and the zone between the two halves of the egg are fluid where "a distinct flow of granules medianward can be observed."

Again, on completion of cleavage Seifríz notes that the two blastomeres become liquid. This statement also fits in with my results. I state (p. 51, '19) that, immediately after cleavage and while the two blastomeres *are still spherical*, the firmness of the cytoplasm persists. Later, when the asters disappear the cytoplasm liquefies and the two blastomeres crowd up against one another. Seifríz noted this last liquid state of the two blastomeres without considering the state prior to it.

I may mention here a possible criticism of the centrifuge method in ascertaining viscosity variations. There are critical stages in the developing asters during which agitation causes their disappearance. This was noted long ago by Wilson. On bringing the eggs to rest the asters reappear and development proceeds normally. I have already discussed this matter fully ('19). The centrifuge and microdissection methods of studying the physical state of protoplasm should serve as valuable checks on one another, if only the investigators in these fields would agree on cooperation.

of the egg, the blastomeres are practically non-adhesive, and they maintain more or less spherical shapes till well on into the later segmentation stages.

#### 6. THE LOCALIZATION OF A MATERIAL, WHICH AFFECTS THE LIFE OF THE UNFERTILIZED STARFISH EGG.

It is well known that immature starfish eggs can be kept in sea water at room temperature for 36 hours or more without disintegrating. That the germinal vesicle or nucleus is responsible for this length of life can be demonstrated by cutting an immature egg in two. The nucleated fragment lasts fully as long as the entire egg. The non-nucleated portion, on the other hand, disintegrates within three to four hours. In mature unfertilized eggs the conditions are quite different. In the mature egg the germinal vesicle has broken down and the nuclear sap has diffused throughout the egg. Loeb ('02) and Mathews ('07) showed that such eggs have a higher rate of oxidation than immature eggs and if left unfertilized, disintegrate within 8 to 10 hours whereas the immature eggs last for days.

The non-nucleated fragment of the mature egg lasts as long as the whole egg, evidently owing to the dispersed nuclear sap of the dissolved germinal vesicle. What is significant is that the nucleated fragment lives no longer than the non-nucleated fragment. Both contain the dispersed nuclear sap, while the nucleated fragment possesses also the definitive mature egg nucleus which is ultimately to become the female pronucleus. Apparently it is the dispersed nuclear sap and not the definitive mature egg nucleus which is chiefly concerned. In the formation of the nucleus of the mature egg we have possibly something analogous to the state of affairs in many Protozoa where the nuclear apparatus consists of a tropho- or macro-nucleus concerned chiefly in the metabolic activities of the cell, and the kineto- or micro-nucleus which has only to do with the reproductive activities. In the starfish egg we may consider the germinal vesicle as a combined tropho- and kineto-nucleus. On the approach of maturation the tropho-nuclear material (nuclear sap) diffuses throughout the egg, leaving behind the kineto-nuclear part, the mature egg nucleus, which gives off the polar bodies to become ultimately the female pronucleus.

The fluid interior of the mature unfertilized egg, if isolated by being made to escape through a tear or the cortex, withstands disintegration for 24 to 36 hours. The presence of even a small part of the original cortex in organic continuity with it causes it to disintegrate in about the same time as an entire mature egg. This would indicate that the reactions which make for disintegration reside chiefly in the cortex. This, together with the fact that the cortex of the egg is necessary for fertilization, would indicate that the cortex is the seat of the initial activation processes of the egg. The relatively inactive central material of the starfish and sand-dollar egg somewhat resembles that of the *Linerges*, the Scyphomedusan, which Conklin ('08) has described. Conklin speaks of "the large cavity in the line of the first cleavage furrow filled with gelatinous or fluid substance, which forms the ground substance of the central area of the unsegmented egg." He found that most of the ground substance escapes into the cleavage cavity and suggested that it is the fluid yoke which is gradually used up in the nourishment of the embryo. The central substance of the *Linerges* egg is probably not strictly analogous with that of the starfish or sand-dollar egg. In *Linerges* cleavage is of a type peculiar to yolk-laden eggs and the central substance escapes during the first cleavage. On the other hand, in the echinoderm egg the nucleus lies well within the central substance of the egg and, upon fertilization, all of the endoplasm is used up in the formation of the cleavage asters and nothing apparently escapes into the early cleavage cavity. We can not, therefore, conclude that the interior of the Echinoderm egg consists of entirely inert material. It lacks certain essential features, but when co-existent with the cortex it plays a full part in the cleavage of the egg.

#### 7. THE LOCALIZATION OF A SUBSTANCE WHICH RENDERS A STARFISH EGG FERTILIZABLE.

Wilson ('03<sup>ab</sup>) in *Cerebratulus* and *Renilla* and Yatsu ('04 and '08) in *Cerebratulus* have shown that non-nucleated fragments of the egg are capable of fertilization only after the germinal vesicle has broken down. With more delicate methods



rendered possible by the microdissection instrument it has been possible to work out this problem in detail and to ascertain to some extent the distribution of the material which renders fertilization possible.

A number of fully grown immature starfish eggs were enucleated by carefully dissecting out their germinal vesicles. None became fertilized when inseminated. In another lot of immature eggs the germinal vesicle was torn while in the egg (Fig. 21). Immediate

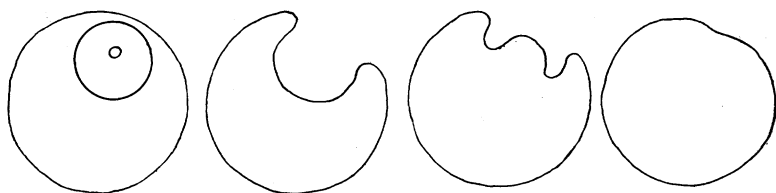


Fig. 21

FIG. 21. A starfish egg whose germinal vesicle is eliminated by puncturing it (cf. Fig. 9). The cytoplasm surrounding this nucleus was also destroyed. This enucleated remnant is nonfertilizable.

dissolution of the nuclear membrane took place with a disintegration of the cytoplasm around the nuclear area. Those eggs which succeeded in forming a protective surface film to prevent spread of the disintegration process subsequently rounded up. Upon insemination none of the eggs showed any sign of being fertilized.

Eggs were then taken with the germinal vesicle in various stages of normal dissolution and cut into nucleated and non-nucleated portions. The eggs may be grouped into stages *b*, *c* and *d*, according to the stage of dissolution of their germinal vesicles, as shown in Fig. 6 (page 323). Whenever the cut passed through the nuclear area during the nuclear stages *b*, *c* and *d*, disintegration always took place, involving all of the nucleated portion and a small part of the non-nucleated piece (Fig. 23 *a*, *b* and *c*). When the cut did not pass through the nuclear area all persisting nucleated portions matured normally and upon insemination formed fertilization membranes and segmented. Of the non-nucleated portions those from eggs in stage *b* are non-fertilizable (Fig. 22). Those from eggs in stage *c* form fertilization membranes upon insemination. Nuclear division also takes place, so that the egg

fragment becomes multi-nucleated but remains unsegmented (Fig. 23-*c*). Non-nucleated fragments of eggs in a later stage (stage *d*) proceed somewhat farther (Fig. 24). The multi-nucleated masses arising from them make several periodic attempts at segmentation (Fig. 24-*c*). Small furrows appear over the surface of the egg, cutting in between the peripherally arranged nuclei.

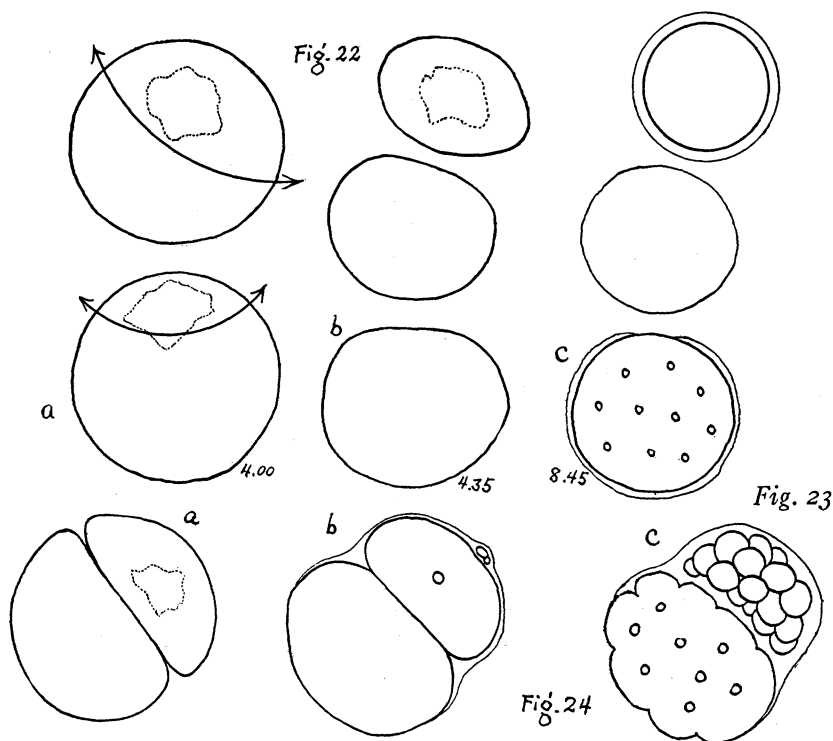


FIG. 22. Starfish egg in stage corresponding to *b* in Fig. 6 cut into two fragments. The non-nucleated fragment contains no material from the germinal vesicle and is nonfertilizable.

FIG. 23. Starfish egg in a later stage corresponding to *c* in Fig. 6 cut through the nuclear area. The cytoplasm in the injured nuclear area disintegrated leaving a non-nucleated fragment, *b*. That the fragment is fertilizable is shown in *c* by the formation of a fertilization membrane and the repeated division of the sperm nucleus. The fragment, however, is unable to segment.

FIG. 24. *a*, starfish egg in stage *d* of Fig. 7 cut into a nucleated and non-nucleated fragment. *b*, both fragments fertilized. The nucleated fragment segmented in the normal way with a number of blastomeres. The non-nucleated fragment became multinucleated and furrows appeared over its surface in an attempt at segmentation.

These furrows then disappear, to reappear again after a short interval. This may occur several times until the egg finally reverts to a spherical shape and remains so. In stage *f* the germinal vesicle has disappeared except for the definitive egg nucleus. Of such eggs any non-nucleated portion down to a certain size is capable of being fertilized and undergoing cleavage.

The above experiments lead one to infer the existence of a substance in the germinal vesicle which, on dissolution of the nuclear membrane, diffuses throughout the cytoplasm. The fertilizability of any egg fragment apparently depends upon the extent of diffusion of this substance. An egg fragment taken when a minimum amount of this substance has diffused into it will allow the sperm nucleus which has entered into it to divide. The presence of a little more of this substance will allow the fragment to undergo abortive segmentation. It is not until a sufficient amount is distributed throughout the egg that any fragment can develop properly.

Mature eggs were now studied, and it was found that any egg fragment in order to be capable of fertilization must contain a portion of the original cortex. The cortex and interior of mature unfertilized eggs were separated according to the method described under heading 4 (Fig. 25 *a* and *b*). The endoplasmic sphere and the cortical remnant were then inseminated. The fragment consisting of the cortical remnant is readily fertilizable and undergoes segmentation (Fig. 25 *b* and *c*). The endoplasmic sphere is non-fertilizable, no matter whether it contains the egg nucleus or not.

That the protoplasm of the endoplasmic spheres has not been irreparably injured in the process of flowing through a small tear in the cortex is shown in the following experiment. Eggs were squashed until the endoplasm protruded as lobate processes, whereupon the pressure on the eggs was lifted and the extrusion allowed to flow back into the egg. Such eggs are fertilizable and are capable of undergoing cleavage. One such case is illustrated in Fig. 26 where the cortex was torn in two places on squashing the egg and two exovates were formed. The nucleated exovate was allowed to pinch itself off. The other exovate flowed back into the remainder of the egg upon insemination (Fig. 26 *b* and *c*). A fairly com-

plete fertilization membrane formed around the egg except at the two torn spots and cleavage followed.

Endoplasmic exovates were also produced which remain connected by a bridge of protoplasm to the collapsed cortical portion

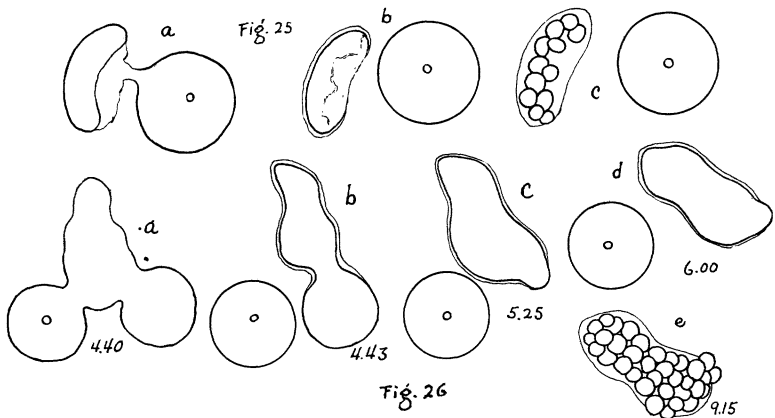


FIG. 25. *a*, nucleated exovate of internal cytoplasm produced by squashing a starfish egg. *b*, fragments inseminated after the endoplasmic sphere was pinched off. Only the ectoplasmic remnant forms a fertilization membrane. *c*, the endoplasmic sphere remains inert and nonfertilizable (cf. Fig. 12).

FIG. 26. *a*, starfish egg squashed producing two endoplasmic exovates. *b*, the nucleated exovate was pinched off. Upon insemination the other exovate drew back into the ectoplasmic remnant which formed a fertilization membrane. *c*, *d* and *e*, the ectoplasmic remnant underwent segmentation showing that the disturbance due to the squashing does not prevent segmentation. The endoplasmic sphere remains inert (*d*).

of the egg. On being inseminated the exovate either is drawn back into the cortical portion as the latter rounds up with the formation of a fertilization membrane or is pinched off, after which it remains as an inert body.

The possibility suggested itself that the substance which renders an egg fertilizable has a tendency to collect in the surface film of an egg and that, if an exovate remained in organic continuity with the egg, this substance might spread to the surface film of the exovate, thus rendering it fertilizable. Endoplasmic exovates were, therefore, produced which remained connected for varying lengths of time with the cortical portion of the egg. Some of the exovates remained connected for as long as fifteen minutes. Before insemination

nation they were pinched off from the cortical portion of the eggs. None developed of those which were separated in such a way that there was no question as to their lacking any of the original cortex of the egg.

An endoplasmic sphere, in order to develop at all, apparently must incorporate in its substance at least a part of the original cortex of the egg. This is shown in Fig. 27. An exovate was

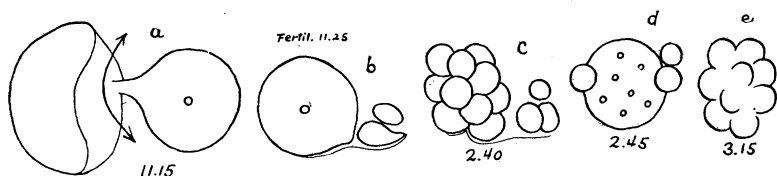


Fig. 27

FIG. 27. *a*, an exovate is produced by squashing and most of the ectoplasmic part is cut away along line of arrow. *b*, the endoplasmic sphere formed itself incorporating a small part of the cortex. Upon fertilization the small cortical region formed a partial fertilization membrane. *c*, many furrows form simultaneously over the surface of the egg showing that it has been fertilized. (Note that the small cortical piece to one side of the egg has segmented in two.) *d*, the egg has reverted into a multinucleated nonsegmented mass except for three blastomere-like bodies which were pinched off. *e*, the fragment is again attempting to segment.

produced by crushing an egg (Fig. 27-*a*). However, before the exovate was set free most of the cortical remnant was cut away, leaving a very small piece which was drawn into the circumference of the endoplasmic sphere. On being inseminated a small shred of the egg membrane lifted off from this remnant, and this was all that constituted the fertilization membrane (Fig. 27-*b*). A sperm on entering this sphere underwent nuclear division several times. This was followed by cleavage furrows which formed on the surface of the egg between the peripheral nuclei and gave to the egg the appearance of a mulberry (Fig. 27-*c*). Some of the furrows deepened sufficiently to pinch off nucleated bodies. A few minutes later the furrows became obliterated and the main body of the egg appeared again as a non-segmented but multinucleated mass (Fig. 27-*d*). This process may occur several times (Fig. 27-*e*). The ability of an exovate to approximate normal segmentation is a function of the amount of the original egg cortex which it incorporates.

The inability of the endoplasmic sphere to develop is not due to the lack of successful sperm entry. Sections show that the sperm enter with ease but they remain unchanged and no asters form about them. In this regard the sperm react exactly as they do when they have entered immature eggs.

There must be something localized in the cortex which is necessary for successful fertilization and development (cf. Lillie, '14, '18). On the evidence presented here we may assume that this substance, originally within the germinal vesicle, diffuses out upon its dissolution and accumulates in the cortex of the egg. It is held in the cortex of the egg and is not carried out in the endoplasmic spheres on crushing the egg. The spheres are, therefore, incapable of being fertilized. Finally, the variation in the ability to segment among exovates containing varying amounts of cortical material indicates that there must also be a definite minimum amount of this substance present in order that an egg fragment may develop.

#### CONCLUSIONS.

1. The nucleus possesses a morphologically definite membrane.
2. Tearing the nucleus results in an immediate change of the nuclear membrane, followed by a disintegration of the cytoplasm surrounding it. This is most striking in the relatively large nucleus (germinal vesicle) of the starfish egg.
3. Injection of the germinal vesicle sap of one egg into the cytoplasm of another egg starts up disintegration processes in the injected area.
4. The mature egg nucleus can be pinched into two fragments. The fragments behave like fluid droplets and will run together when contiguous. Eggs whose nuclei have been operated upon in this manner are capable of normal segmentation.
5. A membrane can be demonstrated adhering to the surface of the unfertilized starfish, sea-urchin and sand-dollar eggs. This egg membrane is most pronounced in the starfish and least of all in the sea-urchin. In the starfish and sand-dollar the membrane can be stripped off without injuring the egg. In the starfish a very delicate egg membrane can be demonstrated investing half-sized

immature eggs. This membrane becomes more pronounced as the eggs reach their full growth and still more so as the egg matures. In the sea-urchin the immature eggs exhibit no trace of a membrane until the eggs begin maturation. In the mature unfertilized sea-urchin egg the membrane has reached a development comparable to that of the half-grown immature egg of the starfish.

6. The egg membrane rises off the surface of the egg upon fertilization and constitutes the fertilization membrane. No appreciable diminution in volume of the egg occurs during this process.

7. An egg, whose membrane has been removed, is fertilizable and segments without a fertilization membrane.

8. The hyaline plasma layer, which forms on the surface of the sea-urchin and sand-dollar egg within ten minutes after fertilization, binds the blastomeres together. In the starfish egg no such layer is formed, and, if the fertilization membrane be removed, the blastomeres tend to fall apart.

9. The fertilizability and approach to normal development of an egg fragment is directly proportional to the amount of a substance which emanates from the germinal vesicle during maturation.

10. The unfertilized mature egg possesses a more solid cortex of appreciable thickness inclosing a highly fluid interior. The fluid interior of the starfish and sand-dollar eggs can be made to ooze out through a tear in the cortex, whereupon it forms a surface film on coming into contact with sea water. In this way the internal and cortical material of the egg can be isolated from one another. Both round up, the internal material immediately and the cortical after some time.

11. Endoplasmic material, possessing a small part of the original cortex, is fertilizable and the approach to normal development is in direct proportion to the amount of cortical material present. The presence of even a small amount of cortical material causes disintegrative changes to set in at about the same time as in a whole egg.

12. The following table gives, for the various kinds of fragments of immature and mature starfish eggs, the length of time that they withstand disintegration when left standing in seawater and also whether they are or are not capable of being fertilized:

	Immature		Mature			
	Nucl. fragm. or entire egg	Non-nucl. fragm.	Nucl. fragm. or entire egg	Non-nucl. fragm.	Nucl. or Non-nucl.	
					Ectoplasmic remnant	Endoplasmic sphere
Longevity in hours...	24-36	2-3	8-10	8-10	8-10	24-36
Fertiliza- bility...	+	-	+	+	+	-
	(when mature)					

As regards longevity it will be seen that the immature egg depends upon its nucleus (germinal vesicle) to prevent disintegration, for a fragment lacking the nucleus disintegrates very quickly. On the other hand, the mature egg, which has become permeated with the nuclear sap of the germinal vesicle, behaves quite differently. The non-nucleated fragment of a mature egg lasts longer than that of an immature egg and it is significant that the presence of the nucleus of the mature egg, which consists of not much more than the chromosomal constituents, has no effect in preventing disintegration.

The long period that the endoplasmic sphere withstands disintegration indicates that the factors which make for disintegration reside chiefly in the original cortex of the mature egg.

In regard to fertilizability it is evident that the substance which renders cytoplasm fertilizable emanates from the germinal vesicle and finally becomes localized in the cortex of the mature egg.

We can, therefore, distinguish three factors in the starfish egg; one affecting longevity, the second affecting disintegration and the third affecting fertilizability. The first and third have been traced to the germinal vesicle of the immature egg. The second is a function of the egg cortex.

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